Biological tumor volume in 18FET-PET before radiochemotherapy correlates with survival in GBM

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Abstract: OBJECTIVE The aim of this prospective longitudinal study was to identify static and dynamic O-(2-[(18)F]fluoroethyl)-l-tyrosine PET ((18)FET-PET)-derived imaging biomarkers in patients with glioblastoma (GBM). METHODS Seventy-nine patients with newly diagnosed GBM were included; 42 patients underwent stereotactic biopsy (unresectable tumors) and 37 patients microsurgical tumor resection. All patients were scheduled to receive radiotherapy plus concomitant and adjuvant temozolomide (RCx/TMZ). (18)FET-PET evaluation using static and dynamic analysis was done before biopsy/resection, after resection, 4 to 6 weeks following RCx, and after 3 cycles of TMZ. Endpoints were survival and progression-free-survival. Prognostic factors were obtained from proportional hazards models. RESULTS Biological tumor volume before RCx (BTVpreRCx) was the most important (18)FET-PET-derived imaging biomarker and was independent of MGMT promoter methylation and clinical prognostic factors: patients with smaller BTVpreRCx had significantly longer progression-free and overall survival (OS). (18)FET time-activity curves (TACs) before treatment and their changes after RCx were also related to outcome; patients with initially increasing TACs experienced longer OS. CONCLUSION BTVpreRCx and TAC represent important (18)FET-PET-derived imaging biomarkers in GBM. Increasing TACs are associated with prolonged OS. The BTVpreRCx is a strong prognostic factor for progression-free survival and OS independent of the mode of surgery. Our data furthermore suggest that patients harboring resectable GBM might benefit from maximal PET-guided tumor resection.

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Original article

Biological tumor volume in ^{18}FET-PET prior to radio-chemotherapy correlates with survival in GBM

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Abstract

Objective: Aim of this prospective longitudinal study was to identify static and dynamic O-(2-[^{18}F]fluorethyl)-L-tyrosine PET (^{18}FET-PET) derived imaging biomarkers in glioblastoma (GBM) patients.

Patients and Methods: 79 patients with newly diagnosed GBM were included; 42 patients underwent stereotactic biopsy (unresectable tumors) and 37 patients microsurgical tumor resection. All patients were scheduled to receive radiotherapy plus concomitant and adjuvant temozolomide (RCx/TMZ). ^{18}FET-PET evaluation using static and dynamic analysis was done prior to biopsy/resection, after resection, 4-6 weeks following RCx and after three cycles of TMZ. Endpoints were survival and progression-free-survival. Prognostic factors were obtained from proportional hazards models.

Results: Biological tumor volume before RCx (BTV_{preRCx}) was the most important ^{18}FET-PET derived imaging biomarker and was independent of *MGMT* promoter methylation and clinical prognostic factors: patients with smaller BTV_{preRCx} had significantly longer progression free (PFS) and overall survival (OS). ^{18}FET time activity curves (TAC) before treatment and their changes after RCx were also related to outcome, patients with initially increasing TAC experienced longer overall survival.

Conclusion: BTV_{preRCx} and TAC represent important ^{18}FET-PET-derived imaging biomarkers in GBM. Increasing TAC are associated with prolonged OS. The BTV_{preRCx} is a strong prognostic factor for PFS and OS independent of the mode of surgery. Our data furthermore suggest that patients harbouring resectable GBM might benefit from maximal PET-guided tumor resection.

Abbreviations: BG, background; BTV, biological tumor volume; CT, computed tomography, ^{18}FET-PET, O-(2-[^{18}F]fluorethyl)-L-tyrosine positron emission tomography; FLAIR, fluid-attenuated inversion recovery; GBM, glioblastoma; Gd gadolinium; Gd+, gadolinium enhanced; HR, hazard ratio; KPS, Karnofsky performance score; LBR, Lesion to brain ratio, *MGMT*, O^6-methylguanyl-DNA-methyltransferase; MRI, magnetic resonance imaging; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; ROC, receiver operating characteristics; RCx, radio/chemotherapy; ROI, region of interest; RT, radiotherapy; SN, sensitivity; SP, specificity; TAC, time activity curve; TMZ, temozolomide, VOI, volume of interest.

Introduction

Glioblastoma (GBM) is a fatal intrinsic brain tumor with median survival times of 11-15 months[1]. Current standard of care includes resection in combination with radiotherapy plus concomitant (RCx) and adjuvant chemotherapy using temozolomide (RCx/TMZ)[2]. Evaluation of imaging biomarkers derived from positron emission tomography (PET) and MRI might improve prognostic evaluation, adjustment of treatment strategies and monitoring of treatment response[3-5]. So far, PET is mainly used in the setting of differential-diagnosis of suspected gliomas, identification of anaplasia or malignant progression[6, 7]. In addition, it could modify/improve radiation treatment planning in combination with MRI[8]. Retrospective data have indicated that the biologically tumor volume (BTV) before radiation therapy as defined by amino-acid PET might correlate with poor outcome in glioblastoma patients: Those with smaller BTVs before radiation did significantly better[9]. The current prospective study was conducted to elucidate the prognostic relevance of BTV before radiation plus concomitant TMZ as defined by ^{18}FET-PET. It was planned to include two patient cohorts of similar size either undergoing biopsy only or tumor resection. Patients with resectable tumors were considered candidates for microsurgery, whereas patients with eloquent tumor location were candidates for biopsy only. All patients were intended to receive RCx/TMZ according to the EORTC/NCIC protocol[2]. Our hypothesis was that BTV before concomitant radio-chemotherapy would be a prognostic/predictive factor independent of the mode of the surgical procedure. Beyond that primary aim of the study, we performed an explorative analysis of static and dynamic PET variables before/after treatment and their possible correlations with outcome.

Patients and Methods

Study design

This prospective longitudinal multicenter study (www.clinicaltrials.gov NCT01089868) was performed within the context of the German Glioma Network (GGN), a non-interventional consortional prospective study funded by the German Cancer Aid (Deutsche Krebshilfe, 70-3163-Wi 3) (www.gliomnetzwerk.de). Adult patients were eligible if they had a supratentorial GBM with histology being proven by stereotactic biopsy or open tumor resection, no prior history of surgery, radiotherapy or chemotherapy, and a Karnofsky performance score (KPS) ≥70. Exclusion criteria were pregnancy and inability to undergo MRI or ^{18}FET-PET for any reason. Resectable tumors received microsurgical resection (cohort A) and unresectable tumors stereotactic biopsy (cohort B). All patients were scheduled to undergo RCx/TMZ according to the EORTC/NCIC protocol[2] and to have ^{18}FET-PET preoperatively, postoperatively (cohort A only), at 4-6 weeks after RCx in analogy to MRI, and after the first three cycles of adjuvant TMZ (Supplemental Fig. e-1). MRI and PET were obtained at the same time points with the specification that the postoperative MRI performed in cohort A was scheduled within the first 72 h after surgery. The study was designed to elucidate in particular the prognostic role of BTV. We planned to include at least 72 patients equally distributed in both cohorts. Given an accrual period of 36 months and a follow-up time of three years, this sample size would allow identifying biomarkers exhibiting a hazard ratio of 2 or higher with a statistical power of 80%. Additionally, the study explored the prognostic relevance of dynamic ^{18}FET-PET-derived biomarkers. Power calculation, however, did not refer to these analyses.

Inclusion period was from 02/2007 until 01/2010, date of last follow-up evaluation was February 28th 2014. Primary endpoint was overall survival (OS), secondary end point was progression-free survival (PFS).

Standard Protocol Apprrovals, Registrations and Patient Consents

All patients gave written informed consent, and the prospective study protocol was approved by the institutional review board (IRB) of the Ludwig Maximilians University, Munich, Germany (AZ 216/04) as well as the IRB of participating sites.

MRI protocol

MRI was performed at the above-mentioned time-points on 1.5 or 3.0 Tesla scanners (Philips Intera 3T, Andover, MA, USA and Signa HDxt, GE Healthcare, Milwaukee, WI, USA). The standardized sequence protocol comprised an axial diffusion-weighted, an axial T_2-weighted, an axial fluid-attenuated inversion recovery (FLAIR) sequence, and isovoxel (3D) T_1-weighted gradient-echo sequences. Two experienced neuroradiologists (J.L., H.J), blinded for the results of ^{18}FET-PET investigations, evaluated the MRI scans including volumetric analyses of each tumor under investigation. For a detailed description of the MRI protocol please see supplemental part.

^{18}FET-PET protocol

Fifty minute-long dynamic emission recording was initiated after injection of approximately 180 MBq ^{18}FET with either a stand-alone PET (EACT EXACT HR+) or a PET/CT (Biograph) scanner (Siemens Medical Systems) according to a standardized acquisition protocol. Semi-quantitative PET data assessment included the maximal standardized uptake value of the tumor corrected for the mean background activity in the healthy contralateral hemisphere reflecting the lesion-to-brain ratio (LBR_{max}) and the estimated biological tumor volume (BTV) defined by semi-automatic threshold-based calculation of a volume of interest (VOI; LBR ≥ 1.8). As previous studies have reported 1.6 as optimal cut-off for untreated glioma[10] and a cut-off value of 2.0 for pre-treated glioma[11], we considered a threshold of 1.8 to be most appropriate for longitudinal assessment of GBM before and after therapy.

Evaluation of dynamic PET recordings was assessed according to our standardized procedure[6]. Resulting time-activity-curves (TAC) were classified into two groups: i) decreasing TAC with SUV showing an early peak within the first 20 min followed by a constant descent thereafter or ii) increasing TAC with SUV constantly ascending or followed by a plateau (for further details, see supplement (Appendix e-1)).

Histology and MGMT analysis

Histological diagnosis was made centrally (H.K.) according to the WHO classification [12]. *MGMT* analysis was performed using methylation-specific PCR[13].

Statistical analysis

Reference point of the study was the date of surgery. Endpoints were death and date of tumor progression. All patient files were reviewed centrally (JCT and MW). Statistical analysis was performed using the SPSS 17.0 software package. PFS and OS were analysed by the Kaplan-Meier method. Comparison of survival curves was done with the two-sided log rank test. Categorical variables were analyzed with the chi-square and continuously scaled variables with the Wilcoxon test. Wilcoxon test for related samples was used to analyze changes of static/dynamic FET-measures over time. Correlation between continuously scaled variables was described with Pearson's correlation coefficient. Potential prognostic factors were identified using proportional hazards models. Parameters which were significant in univariate analyses were included in multivariate models. The optimized model contained only variables that were significantly associated with the endpoint of interest after adjustment for the effects of other variables included in the model. Receiver operating-characteristic (ROC) curve analysis identified cut-off values of continuously scaled imaging biomarkers of interest.

Results

Study population

92 patients were screened for study entry, 79 patients fulfilled the inclusion criteria (Supplemental Fig. e-2): 42 were assigned to cohort A (resection) and 37 to cohort B (biopsy). Age, initial KPS, gender distribution, the frequency of *MGMT* promoter methylation and the applied treatment did not differ in both cohorts. Patient characteristics are summarized in (Supplemental Tab.e-1).

On the date of last follow-up, 70 patients had died (37 in cohort A, 33 in cohort B). Two deaths were not GBM-associated and handled as censored events at the time of their last follow up. Two patients were lost to follow-up. 5 patients are still alive (median follow-up: 48.6 months). 76 patients experienced tumor progression (40 patients in cohort A and 36 patients in cohort B). 64 patients underwent a complete course of RCx, 12 patients did not undergo RCx for various reasons, and in 3 patients RCx was disrupted because of progressive clinical deterioration. 31 patients received 3 cycles of TMZ and thus completed the PET study protocol as planned. The frequency of incomplete or not begun RTCx course was slightly higher in the biopsy group (14 vs 24%, p=0.5). Overall, median PFS was 7.6 months (95 CI % 4.2-11.0) and median OS was 13.5 months (95%CI 10.9-16.7). PFS and OS did not differ significantly between both cohorts. In those undergoing a complete course of RCx, however, OS was longer after surgery than after biopsy (median OS: 16.7 vs. 13.4, p=0.02).

Median initial BTV was 23.8 ccm, median initial LBR_{max} was 3.6; nearly identical values were seen in both cohorts (Table 1). Initial TAC was decreasing in 59/74 tumors and increasing in 15/74 tumors. In five tumors, TAC data were not available.

MRI-based volumetric estimations of the Gd+volume including necrotic/cystic areas and the Gd+volume alone were 35.7 ccm (median) and 13.5 ccm, respectively, while T2/FLAIR volume accounted for 75.0 ccm. The volume distibution was as follows: T2/FLAIR > Gd+volume including necrotic/cystic parts > BTV > Gd+ volume alone.

BTV before RCx

Initial BTV gained prognostic relevance for OS in the biopsy group (p=0.002)**:** Those with smaller BTV did better. In the surgery group, BTV was associated with outcome only after surgery (OS: p=0.002; PFS: p=0.02). In order to determine the potential significance of BTV and SUV_{max}/BG before RCx (BTV_{preRCx} and $SUV_{max}/BG_{preRCx,}$ Tab. 1), post-surgical values of cohort A and the initial values of cohort B were analysed together. BTV before radiotherapy (referred to as BTV_{preRCx} in the following) showed a strong association with survival. According to ROC analyses, the cut-point of BTV_{preRCx} was estimated to be 9.5 ccm (sensitivity (SN): 64%; specificity (SP) 70%). Median OS (PFS) for those with a BTV_{preRCx} below 9.5 ccm was 17.5 (8.8) months and 10.7 (3.9) months for those with a BTV_{preRCx} above 9.5, p=0.002 and p=0.08 (Fig. 1 A-B). The prognostic impact of BTV_{preRCx} was independent of the applied mode of surgery and remained statistically significant in each of the cohorts. The prognostic/predictive impact of BTV_{preRCx} pertained also in the subgroup of 64 patients which completed the course of RCx (OS: p=0.007, PFS: p=0.13). Median BTV_{preRCx} was significantly smaller in the resection group than in the biopsy group (0.8 and 20.1, p<0.0001); however, an overlapping distribution of the BTV_{preRCx} between the cohorts exists.

In those with a BTV_{preRCX} below 9.5 ccm, the median Gd+ volume and Gd+volume including necrotic areas was 0.61 ccm (range: 0-29.6 ccm) and 1.81 ccm (range 0.0-58.9 ccm), respectively. The corresponding values for those with a BTV_{preRCX} above 9.5 ccm were 12.2 ccm (range 0.4-69.5 ccm) and 24.3 ccm (range 0.46-95.9 ccm), respectively. A similar distribution could be observed in biopsy group compared to resection group.

In MRI before RCx, a smaller Gd+volume (without necrotic or cystic parts) was also associated with prolonged OS (p=0.006); Gd+volume plus necrotic tumor parts and T2/FLAIR estimated volume did not gain prognostic impact.

For patient examples of BTV in comparison to Gd+ volume , please see Figure 2.

LBR_{max} and TAC before RCx

Initial LBR_{max} values showed no association with PFS or OS in cohort A or B either considered separately or pooled. However, those with lower LBR_{max}($LBR_{max\text{-}preRCx}$) values experienced longer PFS and OS. According to ROC analysis, the cut-point for $LBR_{max\text{-}preRCx}$ was 2.9 for (SN 68%; SP 73%; p<0.0001, HR 2.9, for OS and p=0.01, HR 2.0, for PFS) (Fig. 1 C-D).

The initial TAC pattern was associated with prognosis: patients exhibiting increasing TAC experienced longer OS (29.7 vs. 12.5 months; p=0.02, HR 2.1, Fig. 1E) and longer PFS (11.9 vs. 5.8 months; p=0.05, HR 1.8, Fig. 1F). LBR_{max}, BTV, and TAC were not correlated with age, KPS or *MGMT* promoter methylation status**.** LBR_{max}values correlated positively with the corresponding BTV (Pearson's R= 0.6, p<0.001).

BTV, $LBR_{max}$and TAC after RCx

$LBR_{max\text{-}preRCx}$ and BTV_{preRCx} decreased after RCx from 3.7 to 2.4 (p<0.0001) and from 28.8 to 12.4 ccm (p<0.0001). No further decrease was seen after 3 cycles of TMZ (Imaging biomarker dynamics are summarized in Table 1). Absolute and relative reduction of $LBR_{max\text{-}preRCx}$ and BTV_{preRCx} after RCx did not relate to PFS and OS. Out of 53 patients in whom PET after RCx was performed, TAC was available in 49 patients (Supplemental Fig. e-2): TAC remained decreasing in 18 patients, changed from decreasing to increasing in 16 patients, remained increasing in 6 patients and changed from increasing to decreasing in 5 patients.

A change from decreasing to increasing TAC was associated with longer PFS compared to patients in whom TAC pattern remained decreasing (6.5 vs. 3.4 months; p=0.017). Patients with a persistently increasing TAC had a longer PFS than those in

whom the pattern changed from increasing to decreasing (16.7 vs. 10.6 months, p=0.4, Figure 3).

Multivariate analyses

A methylated *MGMT* promoter status, higher initial KPS, young age, increasing initial TAC, smaller BTV_{preRCX} and $LBR_{max\text{-}preRCx}$ values, and smaller Gd+volume were favorable prognostic/predictive co-variates for OS in one variable models. For PFS, it was a methylated *MGMT* promoter status, higher initial KPS, increasing $TAC_{postRCX}$, lower BTV_{preRCX} and Gd+volume. Multivariately, small BTV_{preRCx}, increasing initial TAC and *MGMT* promoter methylation were associated with both longer PFS and OS. Additionally, higher KPS values were associated with longer OS. Results of univariale and multivariate models are detailed in Table 2. Nearly identical results were obtained for those undergoing a complete course of RCx (data not given, available on request).

Discussion

In the current prospective study, the biological tumor volume prior to radio-chemotherapy (BTV_{preRCx}) turned out to be a powerful ^{18}FET-PET-derived imaging biomarker: patients with smaller $BTV_{preRCx,}$ either after biopsy or tumor resection, did significantly better in terms of OS and PFS, which remained true after adjustment for the effects of treatment, *MGMT* promoter methylation, and other patient- and tumor related factors. This finding confirms our initial hypothesis. In the biopsy group, the initial BTV (equivalent to the BTV_{preRCx} in this group) gained prognostic relevance whereas in the resection group this was detectable only for the postresection BTV (which is BTV_{preRCx} in this group). Since minimal BTV_{preRCx} is strongly linked to OS, maximal surgical reduction of the BTV seems to be essential in resectable tumors.

This might explain why the inital BTV in the surgery group did not gain any impact on outcome in contrast to the finding in the biopsy group.

Interestingly, removal of only necrotic parts of the tumor volume seems not to alter the prognosis as MRI based Gd+ volume was also significantly associated with OS while Gd+ volume including necrotic/cystic area and T2/FLAIR volume was not. This is in line with data from a previous multicenter study with MRI as the only imaging modality[14].

The optimal threshold for favorable/unfavorable BTV after biopsy/surgery still needs elucidation and could not be precisely resolved by the design of this and other studies[9]. However, a post-resection GD+volume of approximately 0 ccm in the surgery group can harbour a BTV_{preRCx} of up to 9.5 ccm. This might explain that survival in both cohorts was rather similar even though BTV_{preRCx} was lower in the surgery group. Only for those patients completing RC_X, a moderate survival benefit was detected after surgery. This implies that BTV-based prognostic evaluation will refine indications for open tumor resection and biopsy in glioblastoma patients: in lesions which are not amenable for a safe and significant surgical reduction of the biologically relevant active tumor volume (which will not be achieved by removal of necrotic tissue only), the role of surgery might be questionable whereas patients with large BTVs and surgically accessible tumors might be good candidates for tumor resection.

The association of the $LBR_{max\text{-}preRCx}$ and outcome, although positively correlated with the respective BTV, was more heterogeneous; its impact was clearly dominated by the BTV_{preRCx} in multivariate models.

The optimal time point for PET-guided assessment of treatment response after RCx of GBM patients remains unknown. We demonstrate that both BTV and LBR_{max} significantly decreased six weeks after RCx; however, with no prognostic threshold for these static measures. Unspecific ^{18}FET uptake in treatment-induced reactive gliosis

might have confounded the discrimination between responders and non-responders in the current study[15]. Recently published studies showed conflicting results: a significant influence was found 6-8 weeks after RCx, however, this was not confirmed after a longer observation time[16,17]. Whether a PET scan 7-10 days after RCx is more reliable for the evaluation of treatment response has yet to be elucidated[9]. Yet, short time evaluation could interact with occurrence of pseudoprogression: as imaging was conducted after 6-8 weeks in our study, only two patients showed pseudoprogression in MRI. Interestingly, there was no correlating increase in BTV in those patients. This small number is in line with a recent publication examining the incidence of pseudoprogression[18]. Thus, future prospective studies should resolve uncertainties concerning appropriate time points for treatment response assessment by static ^{18}FET-PET analysis.

We have previously demonstrated the prognostic value of ^{18}FET kinetics in recurrent high-grade glioma[19]. Based on the present study, analysis of TAC and their changes during the disease course offers an additional imaging biomarker for early prognostic evaluation and assessment of treatment response. Initial TAC and TAC changes after RCx had prognostic influence on outcome. These results seem to contrast findings of a recent study where no influence was detected for several kinetic measures[17]. Notably, in the latter study comprising a small study population, TAC were evaluated in differently defined ROIs: pretherapeutic TAC were assessed within the tumor ROI corresponding to the target volume of radiotherapy, which was subsequently transferred to the coregistered slices of the follow-up PET for the analysis of posttherapeutic TAC. Therefore, the method of dynamic analysis, namely the ROI definition, seems to be essential for the assessment of the prognostic value. Furthermore, it remains subject to further investigations to elucidate whether tumors exhibiting increasing TAC differ in biology and/or vascularity from those with a decreasing pattern. FET uptake is influenced by the expression of amino acid

transporters as well as tumor perfusion[20-22], however, the underlying mechanisms concerning the slope of the TAC remain to be explored.

Our study has limitations: first, we did not apply RANO criteria since the study started prior to their publication[23, 24]. Second, evaluation of TAC was based on a relatively small sample size and deserves further evaluation**.**

Altogether, ^{18}FET-PET-derived imaging biomarkers before and after concomitant radio-/chemotherapy in GB are powerful tools to obtain prognostic information for outcome in newly diagnosed GBM. BTV as delineated by ^{18}FET-PET before RCx/TMZ correlates with PFS and OS. Tumor uptake kinetic of ^{18}FET is associated with PFS. These findings might help to individualize therapy and response evaluation in forthcoming clinical investigations.

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References

1. Koshy M, Villano JL, Dolecek TA, et al. Improved survival time trends for glioblastoma using the SEER 17 population-based registries. Journal of neuro-oncology 2012;107:207-212.
2. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459-466.
3. Li Y, Lupo JM, Parvataneni R, et al. Survival analysis in patients with newly diagnosed glioblastoma using pre- and postradiotherapy MR spectroscopic imaging. Neuro-oncology 2013;15:607-617.
4. Holodny AI, Makeyev S, Beattie BJ, Riad S, Blasberg RG. Apparent diffusion coefficient of glial neoplasms: correlation with fluorodeoxyglucose-positron-emission tomography and gadolinium-enhanced MR imaging. AJNR American journal of neuroradiology 2010;31:1042-1048.
5. Dhermain FG, Hau P, Lanfermann H, Jacobs AH, van den Bent MJ. Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. Lancet neurology 2010;9:906-920.
6. Jansen NL, Graute V, Armbruster L, et al. MRI-suspected low-grade glioma: is there a need to perform dynamic FET PET? European journal of nuclear medicine and molecular imaging 2012;39:1021-1029.
7. Galldiks N, Stoffels G, Ruge MI, et al. Role of O-(2-18F-fluoroethyl)-L-tyrosine PET as a diagnostic tool for detection of malignant progression in patients with low-grade glioma. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2013;54:2046-2054.
8. Gotz I, Grosu AL. [(18)F]FET-PET Imaging for Treatment and Response Monitoring of Radiation Therapy in Malignant Glioma Patients - A Review. Frontiers in oncology 2013;3:104.
9. Piroth MD, Holy R, Pinkawa M, et al. Prognostic impact of postoperative, pre-irradiation (18)F-fluoroethyl-l-tyrosine uptake in glioblastoma patients treated with radiochemotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 2011;99:218-224.
10. Pauleit D, Floeth F, Hamacher K, et al. O-(2-[18F]fluoroethyl)-L-tyrosine PET combined with MRI improves the diagnostic assessment of cerebral gliomas. Brain 2005;128:678-687.
11. Mehrkens JH, Popperl G, Rachinger W, et al. The positive predictive value of O-(2-[18F]fluoroethyl)-L-tyrosine (FET) PET in the diagnosis of a glioma recurrence after multimodal treatment. Journal of neuro-oncology 2008;88:27-35.
12. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97-109.
13. Grasbon-Frodl EM, Kreth FW, Ruiter M, et al. Intratumoral homogeneity of MGMT promoter hypermethylation as demonstrated in serial stereotactic specimens from anaplastic astrocytomas and glioblastomas. Int J Cancer 2007;121:2458-2464.
14. Kreth FW, Thon N, Simon M, et al. Gross total but not incomplete resection of glioblastoma prolongs survival in the era of radiochemotherapy. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2013;24:3117-3123.
15. Jansen NL, Suchorska B, Schwarz SB, et al. [18F]fluoroethyltyrosine-positron emission tomography-based therapy monitoring after stereotactic iodine-125 brachytherapy in patients with recurrent high-grade glioma. Molecular imaging 2013;12:137-147.
16. Galldiks N, Langen KJ, Holy R, et al. Assessment of treatment response in patients with glioblastoma using O-(2-18F-fluoroethyl)-L-tyrosine PET in comparison to MRI. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2012;53:1048-1057.
17. Piroth MD, Liebenstund S, Galldiks N, et al. Monitoring of radiochemotherapy in patients with glioblastoma using O-(2-(1)(8)Fluoroethyl)-L-tyrosine positron emission tomography: is dynamic imaging helpful? Mol Imaging 2013;12:388-395.
18. Radbruch A, Fladt J, Kickingereder P, et al. Pseudoprogression in patients with glioblastoma: clinical relevance despite low incidence. Neuro-oncology 2014.
19. Popperl G, Kreth FW, Herms J, et al. Analysis of 18F-FET PET for grading of recurrent gliomas: is evaluation of uptake kinetics superior to standard methods? Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2006;47:393-403.
20. Langen KJ, Hamacher K, Weckesser M, et al. O-(2-[18F]fluoroethyl)-L-tyrosine: uptake mechanisms and clinical applications. Nuclear medicine and biology 2006;33:287-294.
21. Stockhammer F, Plotkin M, Amthauer H, van Landeghem FK, Woiciechowsky C. Correlation of F-18-fluoro-ethyl-tyrosin uptake with vascular and cell density in non-contrast-enhancing gliomas. J Neurooncol 2008;88:205-210.
22. Wyss MT, Hofer S, Hefti M, et al. Spatial heterogeneity of low-grade gliomas at the capillary level: a PET study on tumor blood flow and amino acid uptake. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2007;48:1047-1052.

23. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1963-1972.
24. Vogelbaum MA, Jost S, Aghi MK, et al. Application of novel response/progression measures for surgically delivered therapies for gliomas: Response Assessment in Neuro-Oncology (RANO) Working Group. Neurosurgery 2012;70:234-243; discussion 243-234.

Table 1. BTV, LBR and TAC values

	Value	Initial PET	PET after resection	PET after RCx	PET after TMZ
All	LBR_{max}				
	mean	3.7		2.4 (*)	2.3 (*)
	median	3.6		2.3	2.2
	SD	1.03		0.81	0.94
	BTV (ccm)				
	mean	28.8		12.4 (*)	11.7 (*)
	median	23.8		4.8	5.4
	SD	22.8		19.7	16.78
	increasing TAC (patients)	15		26	7
	decreasing TAC (patients)	59		23	16
	Gd+ volume MRI (+necrosis)				
	mean	42.9			
	median	33.9		-	-
	SD	37.0			
	Gd+ volume MRI (-necrosis)				
	mean	17.4			
	median	13.5		-	-
	SD	14.8			
	T2/FLAIR volume MRI				
	mean	86.1			
	median	75.0			
	SD	51.7			
Cohort A	LBR_{max}				
	mean	3.8	2.1 (*)	2.1 (*)	2.0
	median	3.7	2.0	2.1	2.0
	SD	1.15	1.45	0.75	0.94
	BTV (ccm)				
	mean	31.5	3.1 (*)	4.6 (*)	10.1
	median	24.8	0.75	1.8	1.4
	SD	26.07	5.87	7.87	17.71
	increasing TAC (patients)	8	15	14	8
	decreasing TAC (patients)	34	16	9	4
	Gd+ volume MRI (+necrosis)				
	mean	53.7	8.6(*)		
	median	46.3	0.6	-	-
	SD	43.2	13.8		
	Gd+ volume MRI (-necrosis)				
	mean	21.5	2.1 (*)		
	median	21.3	0.0	-	-
	SD	15.32	3.76		
	T2/FLAIR volume MRI				
	mean	104.7	25.1(*)		
	median	87.9	18.4		
	SD	57.5	21.8		
Cohort B	LBR_{max}				
	mean	3.6		2.8 (*)	2.9 (**)
	median	3.5		2.5	2.9
	SD	0.86		0.72	0.67
	BTV (ccm)				
	mean	25.8		20.0 (**)	13.9 (**)
	median	21.8		10.7	13.8
	SD	17.5		24.34	15.97
	increasing TAC (patients)	8		9	8
	decreasing TAC (patients)	29		16	3
	Gd+ volume MRI (+necrosis)				
	mean	32.5			
	median	24.9		-	-
	SD	26.2			
	Gd+ volume MRI (-necrosis)				
	mean	13.7			
	median	11.9		-	-
	SD	13.47			
	T2/FLAIR volume MRI				
	mean	68.7			
	median	66.5			
	SD	38.78			

Abbreviations: LBR_{max}, maximal lesion-to-brain ratio, BTV, biological tumor volume (determined by ^{18}FET-PET), TAC, time activity curves, Gd+, gadolinium enhanced. Legend: Wilcoxon test: *p <0.0001, **p<0.05; ()= comparison with values from the first column

Table 2. Results univariate/multivariate analysis

Univariate Analysis of OS and PFS						
	Progression			Death		
Factor	Relative Risk	95% CI	*P*	Relative Risk	95% CI	*P*
MGMT promoter						
Unmethylated	1.00			1.00		
Methylated	0.40	0.23-0.71	.002	0.35	0.21-0.61	<.0001
KPS*	0.97	0.94-1.00	.03	0.95	0.93-0.98	<.0001
Age*	1.00	0.99-1.03	.16	1.03	1.01-1.05	.005
Mode of surgery						
Resection	0.86	0.52-1.43		0.73	0.45-1.17	
Biopsy	1.00		.72	1.00		.19
Initial TAC						
increasing	1.00			1.00		
decreasing	1.83	0.98-3.41	.06	2.05	1.10-3.83	.02
$TAC_{POSTRCx}$						
increasing	0.46	0.25-0.84	.01	0.9	0.74-1.30	.9
decreasing	1.00			1.00		
BTV_{PRERCx}*	1.02	1.01-1.04	.03	1.03	1.01-1.05	<.0001
$LBR_{max\text{-}PRERCx}$*	1.19	0.94-1.50	.15	1.38	1.12-1.69	.002
Gd+MRI Volume*	1.02	1.00-1.05	.03	1.03	1.01-1.05	.006

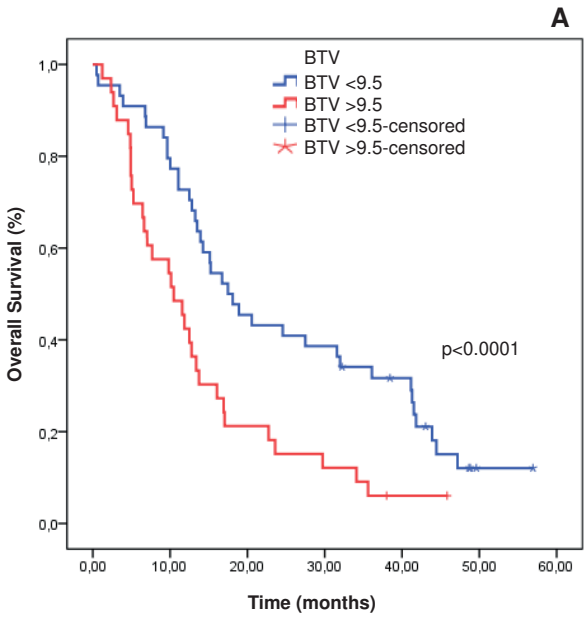
NOTE. * continuously scaled ; KPS 10 points increment

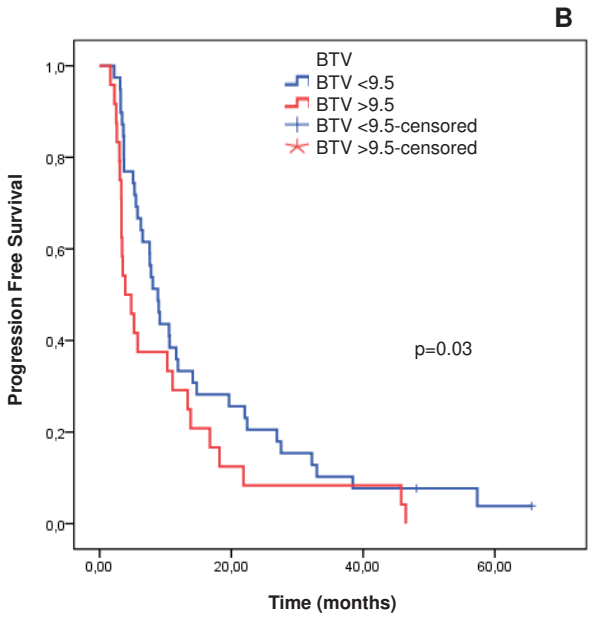
Abbreviations: MGMT: O^6-methylguanyl-DNA-methyltransferase, KPS, Karnofsky performance score, TAC, time activity curves, BTV, biological tumor volume (determined by ^{18}FET-PET), LBR_{max}, maximal lesion-to-brain ratio, preRCX, before radiochemotherapy, postRCx, after radiochemotherapy, Gd+, Gadolinium enhanced

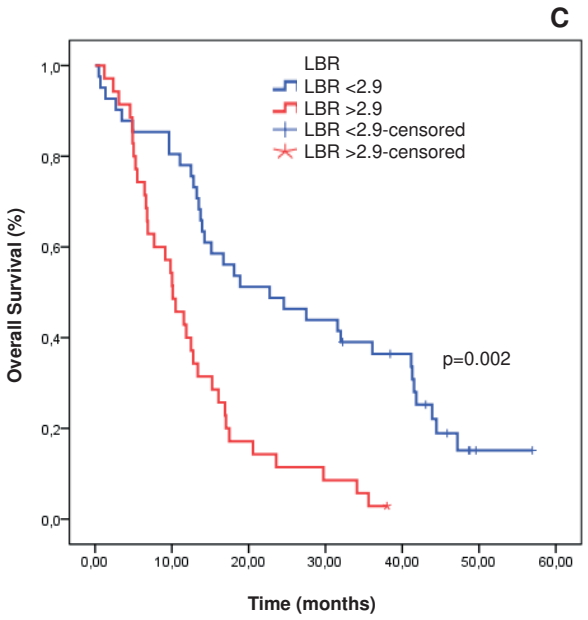
Multivariate Analysis of OS and PFS						
	Progression			Death		
Factor	Relative Risk	95% CI	*P*	Relative Risk	95% CI	*P*
MGMT promoter						
Unmethylated	1.00			1.00		<.0001
Methylated	0.45	0.27-0.80	.007	0.27	0.15-0.49	
KPS*	0.98	0.95-1.0	.06	0.96	0.93-0.98	<.0001
Initial TAC						
increasing	1.00					
decreasing	1.92	0.98-3.76	.06	2.03	1.05-3.90	.035
BTV_{PRERCx}*	1.02	1.00-1.04	.018	1.03	1.01-1.05	<.0001

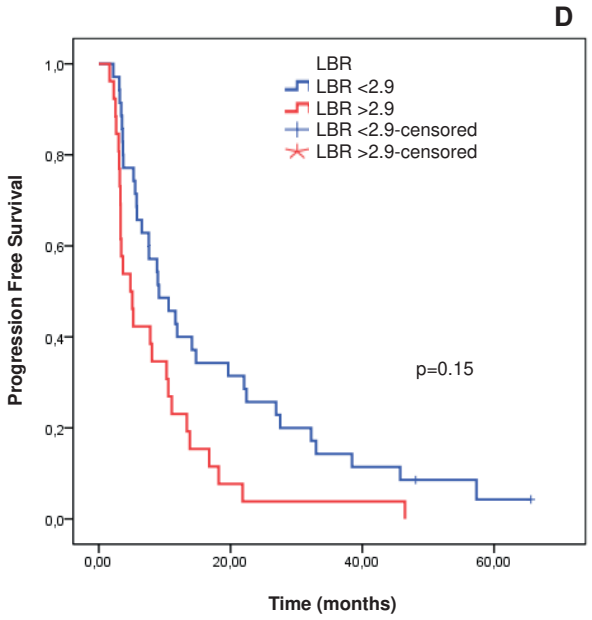
NOTE. * continuously scaled; KPS 10 points increment, Abbreviations: ns, not significant, MGMT: O^6-methylguanyl-DNA-methyltransferase, KPS, Karnofsky performance score, TAC, time activity curves, BTV, biological tumor volume (determined by ^{18}FET-PET), preRCX, before radiochemotherapy

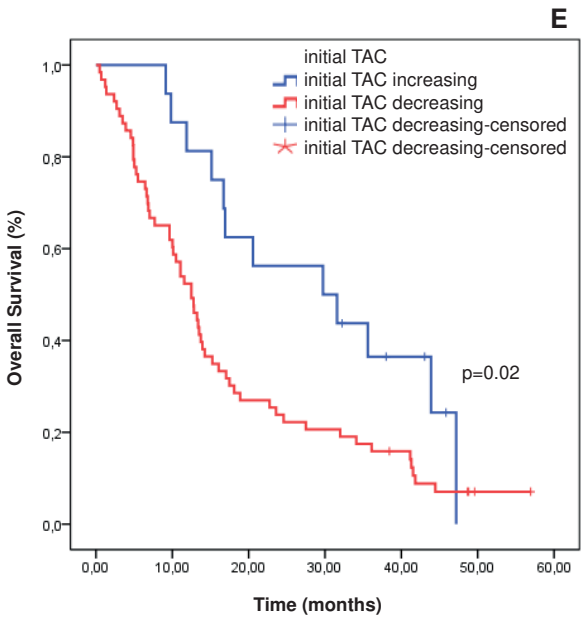
Figure 1 A-F: Association of BTV_{preRCx} (A/B), $LBR_{max\text{-}preRCx}$ (C/D) and initial TAC (E/F) with OS and PFS

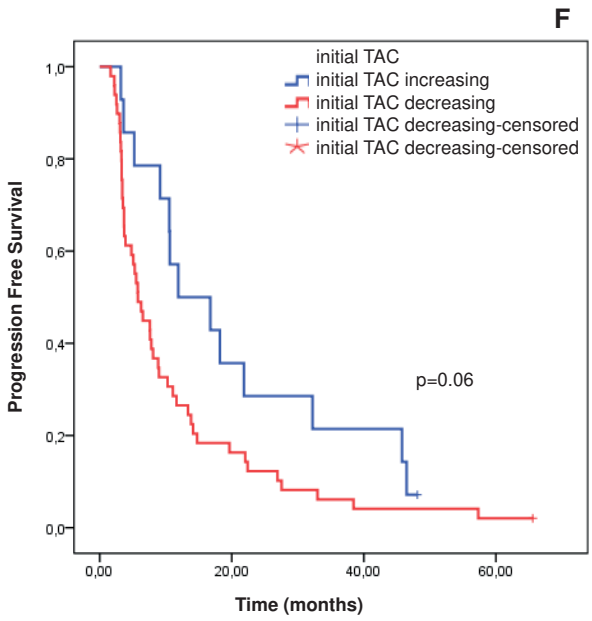












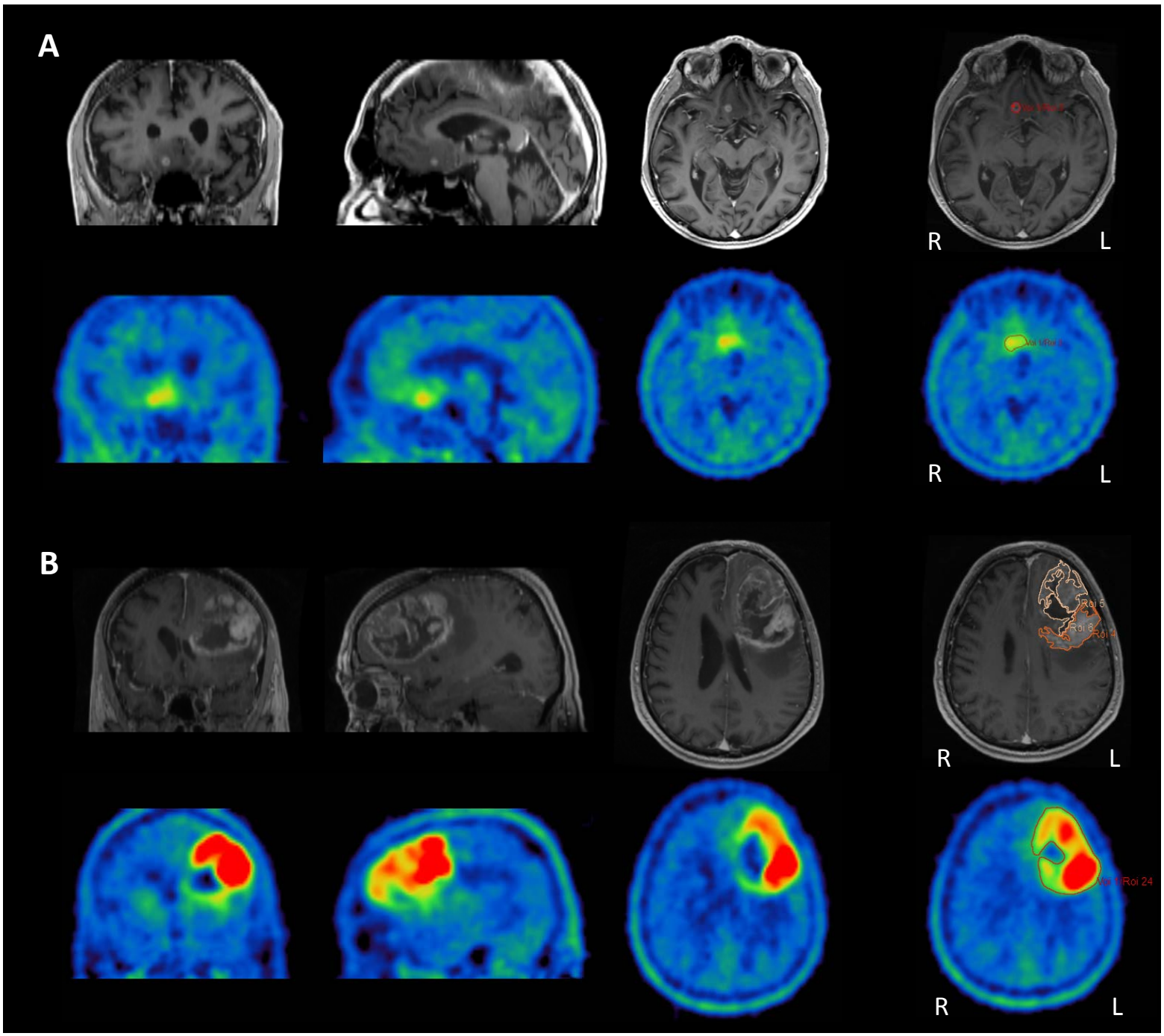


Fig. 3: Patient examples: (A) Patient with a favourable outcome (OS xx months) with an initial BTV of xx ccm, Gd+ volume (-necrosis) of xx ccm. (B) Patient with an unfourable outcome (OS xx months) with an initial BTV of xx ccm and Gd+ volume (- necrosis) of xx ccm.